

# Effect of Supercritical Carbon Dioxide Pressurized with Helium on Solute Solubility during Supercritical Fluid Extraction

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Carbon dioxide cylinders, pressurized with a helium headspace, find routine use as a solvent in analytical supercritical fluid extraction (SFE). In this study, the solubilities of soybean oil in neat supercritical carbon dioxide (SC-CO<sub>2</sub>) and CO<sub>2</sub> with entrained helium were measured at 70 °C and three different pressures. The oil solubility was found to be substantially diminished when the CO<sub>2</sub>/He source was used, presumably by the presence of small amounts of helium dissolved in the extraction fluid. The recorded reductions in solubility (30–50 wt %) should be taken into account in performing and optimizing SFE with helium-pressurized cylinders.

Cylinders containing carbon dioxide pressurized with the aid of a relatively insoluble gas such as helium have been utilized extensively for analytical supercritical fluid extraction (SFE) over the last several years. The rationale for pressurizing the carbon dioxide source by this method is that it permits fluid delivery devices (e.g., syringe or booster pumps) to operate more efficiently by assuring adequate liquification of gas in the pump cylinder or head without the need for external circulating cooling baths. Such gas sources have seen routine use with a variety of commercial instruments and are a item of commerce.

Previous investigators<sup>3,4</sup> have noted the effect of using He-pressurized CO<sub>2</sub> sources on the retention time reproducibility of solutes in supercritical fluid chromatography (SFC). More recently, Raynie and Delaney<sup>5</sup> have reported that He entrained in CO<sub>2</sub> reduces the extraction rate of cholesterol in SFE relative to those values observed with neat CO<sub>2</sub>. Raynie and Delaney suggested that this effect is due to a suppression of the cholesterol's solubility in the potential CO<sub>2</sub>/He mixture when it is pressurized for use in SFE.

To verify the above hypothesis, we have conducted independent experiments on the solubility of soybean oil in SC-CO<sub>2</sub>, with and without He pressurization, in our laboratory. Soybean oil solubility in SC-CO<sub>2</sub> as a function of temperature and pressure has been extensively studied by several researchers,<sup>6–9</sup> and provides a good test case for observing the effect of entrained

helium on the solubility of a lipid solute which exhibits a high solubility in SC-CO<sub>2</sub>. In addition, soybean oil emulates the behavior of a typical "fat" in SC-CO<sub>2</sub>; hence if an appreciable difference does occur in the effective solubility of soybean oil in CO<sub>2</sub> versus a CO<sub>2</sub>/He mixture, then the projected time of extraction as well as quantity of CO<sub>2</sub> required for exhaustive delipidation of fat/oil-containing samples would be effected. Such a result is important to the analyst in optimizing the SFE parameters and in this projected high-volume use of analytical SFE.<sup>10</sup>

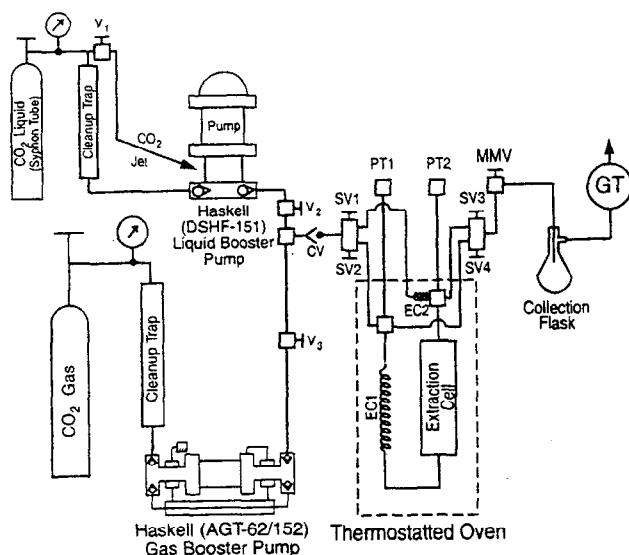
## EXPERIMENTAL SECTION

Our interest in the above phenomena was catalyzed by initial experiments on an Applied Separations (Allentown, PA) Spe-ed supercritical fluid extraction unit. This instrument is based on a earlier extractor design developed in our laboratory<sup>11</sup> and incorporates a Haskel, Inc. (Burbank, CA) liquid booster pump to deliver the extraction fluid, CO<sub>2</sub>. Extraction of soya flakes on this unit using a helium-pressurized CO<sub>2</sub> produced a oil solubility (in wt %) that was ~2.5 times less than that previously determined using neat CO<sub>2</sub> at 5000 psi and 70 °C.<sup>12</sup> Oil solubility in the supercritical fluid was calculated from the mass of oil collected per mass of CO<sub>2</sub> passed through the extraction vessel at several intervals in the linear portion of oil extraction curve,<sup>13</sup> and the average of these determinations was used to yield the weight percent of oil in the SC-CO<sub>2</sub>.

To more accurately determine the effect of helium entrainment in SC-CO<sub>2</sub> on the solute (oil) solubility in the SC-CO<sub>2</sub>, a home-built extraction apparatus was constructed, as shown in Figure 1. In this apparatus, two different Haskel air-driven booster pumps were employed to deliver the CO<sub>2</sub>. A Haskel Model AGT 62/152 booster pump was used to deliver gaseous CO<sub>2</sub>, while a Haskel liquid pump, Model DSHF-151, was utilized to pump the liquified CO<sub>2</sub>. In the latter case, a syphon tube liquid CO<sub>2</sub> source, both with and without helium pressurization, was used to supply the liquid booster pump. As shown in Figure 1, this dual fluid

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**Figure 1.** Experimental supercritical fluid extraction unit, permitting the use of two different fluid sources and delivery pumps. Key: V, on/off valves; SV, switching valves; PT, pressure transducer; EC, equilibration coil; MMV, micrometering valve; GT, gas totalizer (dry test meter).

pumping arrangement permitted identical SFEs to be performed on the same extraction apparatus; however, either gaseous, liquid, or liquid CO<sub>2</sub> with entrained helium could be used to perform the SFE.

Welding grade, gaseous and liquified CO<sub>2</sub> obtained from National Welding Supply (Bloomington, IL) were used in our experiments. Helium-pressurized cylinders of liquified CO<sub>2</sub> initially compressed to 1900 psi were also obtained from the above source. The head of the Model DSHF-151 pump was cooled by venting liquified CO<sub>2</sub> onto it to prevent cavitation in the pump head when a liquid CO<sub>2</sub> source without helium pressurization was used.

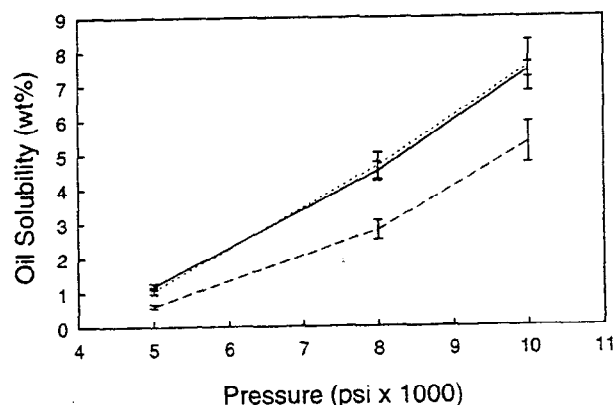
The details of vegetable oil extraction with this type of apparatus have been described previously.<sup>14,15</sup> Typically, 60 g of soybean flakes, contained in a 10 000 psi-rated extraction cell, 61 cm length × 1.75 cm i.d., was utilized for each SFE run. Extractions were conducted for a time period commensurate with establishing a constant oil solubility in the SC-CO<sub>2</sub>. Oil solubilities were calculated from at least three or more consecutive solubility measurements during the same SFE. Recovered oil was measured intermittently by gravimetry, while the CO<sub>2</sub> used in each interval was measured by a dry test meter (Model DTM 200A, American Meter Co., Philadelphia, PA) at ambient conditions.

SFEs were run at 70 °C three different pressures: 5000, 8000 and 10 000 psi. To ensure accurate solubility measurements, triplicate extraction runs were conducted for the three types of fluid delivery systems at each pressure, for a total of 27 individual runs. The resultant solubility data were analyzed with the aid of a Statistix 4.1 software program (Analytical Software, Tallahassee, FL) using multiple regression and analysis of covariance (ANCOVA).

**Table 1**

fluid source	pressure (psi)	wt % oil solubility <sup>a</sup>
CO <sub>2</sub> (g)	5000	1.18 ± 0.09*
	8000	4.50 ± 0.27
	10000	7.45 ± 0.23
CO <sub>2</sub> (l)	5000	1.06 ± 0.09
	8000	4.66 ± 0.38
	10000	7.57 ± 0.75
CO <sub>2</sub> (l)/He(l)	5000	0.61 ± 0.06
	8000	2.80 ± 0.28
	10000	5.53 ± 0.61

<sup>a</sup> Mean ± standard deviation



**Figure 2.** Effect of fluid source on oil solubility as a function of extraction pressure: (—) gaseous CO<sub>2</sub>, (···) liquid CO<sub>2</sub>, and (---) liquid CO<sub>2</sub>/He.

## RESULTS AND DISCUSSION

Results from the measurement of soybean oil solubility in SC-CO<sub>2</sub> and the SC-CO<sub>2</sub>/He mixture as a function of pressure at 70 °C are presented in Table 1. The results are tabulated according to the fluid source/pumping system utilized. Fluid from the CO<sub>2</sub>(g) pumping system were delivered to the extractor via a gas booster pump and utilized no helium in the CO<sub>2</sub> cylinder. The data listed under CO<sub>2</sub>(l) were obtained with liquid booster pump delivery of the CO<sub>2</sub> sans He. The third group of solubility data were determined on the liquid booster pump system using CO<sub>2</sub> that had been pressurized with helium as noted above.

The results in Table 1 show that solubility of soybean oil in SC-CO<sub>2</sub> increases with pressure at 70 °C.<sup>16</sup> Note that, for the CO<sub>2</sub> sources without helium, the solubility values at each specific pressure are almost identical. When the cylinder containing CO<sub>2</sub> pressurized with He is utilized for the SFE, solubility values are decreased by factors of 0.50, 0.62, and 0.73 at 5000, 8000 and 10 000 psi, respectively. This substantial reduction in oil solubility is outside the limit of experimental error as predicted by the standard deviations associated with the respective means of the multiple solubility determinations for each pressure level and particular fluid source.

The variation of oil solubility with pressure is nearly linear over the pressure interval from 5000 to 10 000 psi as confirmed in Figure 2. The slopes associated with the linear least-squares fits to the experimental data yield *r*<sup>2</sup> values of 0.86, 0.97, and 0.94 for the CO<sub>2</sub>(g), CO<sub>2</sub>(l), and CO<sub>2</sub>(l)/He(l) source/delivery systems, respectively. It should be noted that this apparent linear relation-

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ship between weight percent solubility and pressure does not hold for CO<sub>2</sub> over the entire pressure range from ambient to 10 000 psi.<sup>12</sup> Clearly the data show that the utilization of a CO<sub>2</sub>/He fluid source results in lowering the oil solubility in the extraction fluid. Figure 2 shows that, within the limits of experimental error, the determined soybean oil solubility values do not vary with pressure when neat CO<sub>2</sub> is used, regardless of the type pump that is used for fluid delivery.

Our experiments also permitted for the statistical evaluation of the effects of parameters such as the source of extraction fluid, pressure, fluid flow rate, and interaction between extraction fluid and pressure on percent oil solubility. The analysis of the covariance verified that there was a significant effect of the fluid source (type) on the oil solubility in the fluid ( $F_{2,74} = 81.23$ ,  $P = 0.0000$ ). Regression analysis confirmed that both CO<sub>2</sub> sources devoid of helium pressurization yielded statistically equivalent oil solubilities ( $t = 0.30$ ,  $P = 0.7647$ ,  $df = 74$ ) ( $df$ , degrees of freedom); however, these fluid sources gave statistically higher oil solubilities than the CO<sub>2</sub> source having helium ( $t = 2.94$ ,  $P = 0.0043$ ,  $df = 74$  for gaseous CO<sub>2</sub> and  $t = 2.65$ ,  $P = 0.0099$ ,  $df = 74$  for liquid CO<sub>2</sub>). The regression analysis indicated that there was a significant effect of extraction pressure on the resultant oil solubility ( $t = 34.13$ ,  $P = 0.0000$ ,  $df = 74$ ), and ANCOVA indicated a significant interaction between the fluid source and extraction pressure ( $F_{2,80} = 30.64$ ,  $P = 0.0000$ ). The slopes of the lines in Figure 2 are equivalent for both CO<sub>2</sub> sources that did not contain helium ( $t = 0.11$ ,  $P = 0.9094$ ,  $df = 74$ ), however, their slopes were significantly higher than those observed for the helium/CO<sub>2</sub> source ( $t = 6.83$ ,  $P = 0.0000$ ,  $df = 74$  for gaseous CO<sub>2</sub> and  $t = 6.73$ ,  $P = 0.0000$ ,  $df = 74$  for liquid CO<sub>2</sub>). The overall regression equation predicting oil solubility was found to be highly significant ( $F = 590.75$ ,  $P = 0.0000$ ), having an  $R^2$  of 0.9795. The regression equations predicting weight percent oil solubility for the three fluid sources are

$$\text{CO}_2(\text{g}) \text{ \% solubility} = -6.664 + (0.00135)P + (0.240)F \quad (1)$$

$$\text{CO}_2(\text{l}) \text{ \% solubility} = -6.571 + (0.00134)P + (0.240)F \quad (2)$$

$$\text{CO}_2(\text{l})/\text{He}(\text{l}) \text{ \% solubility} = -5.469 + (0.00100)P + (0.240)F \quad (3)$$

where  $P$  is pressure (psi) and  $F$  is ambient flow rate (L/min).

It should be noted that eq 3 also predicts that the fluid flow rate can influence the resultant oil solubility ( $t = 4.72$ ,  $P = 0.0000$ ,  $df = 74$ ), with % oil solubility increasing slightly with pressure.

The influence of dissolved He in liquid CO<sub>2</sub> cylinder sources has also been implicated in a study of flame ionization detector response by Schweighardt and Mathias.<sup>17</sup> These researchers, invoking the vapor/liquid equilibria data of Burfield et al.,<sup>18</sup> suggested that as little as 0.5 wt % (~5 mol %) helium in CO<sub>2</sub> could account for the larger decrease in baseline shift during pressure programming in supercritical fluid chromatography using

a FID. This effect, they argued, was due to the differential adiabatic expansion (Joule-Thomson effect) between SC-CO<sub>2</sub> and SC-He as the carrier fluid is expanded through the column outlet restrictor.

Similarly, Gorner et al.<sup>19</sup> observed considerable differences in retention times for solutes in SFC when helium head pressure CO<sub>2</sub> was used in place of neat CO<sub>2</sub> as a mobile phase. They performed rigorous thermodynamic calculations of the density of the CO<sub>2</sub>/He mixtures for their experimental conditions and predicted a reduction in the fluid density of up to 20% when neat CO<sub>2</sub> is diluted with small quantities of dissolved He. Such an effect could also be influencing the oil solubility measurements reported in this study since the dissolving power of CO<sub>2</sub> is a function of its density. Unfortunately, there are no experimentally determined densities for He/CO<sub>2</sub> mixtures under the conditions employed in this study. Gahrs<sup>20</sup> noted a reduction in caffeine solubility in SC-CO<sub>2</sub> by the addition of small quantities of N<sub>2</sub> or Ar to the CO<sub>2</sub>. This verifies the trends found in our measurements and suggests that further research in this area could lead to a control of extraction selectivity in analytical SFE via compositional changes in the fluid phase.

Schweighardt and Mathias<sup>21</sup> calculated the mole percent of helium dissolved in a liquid CO<sub>2</sub> as a function of pressure for a gas cylinder kept at typical room temperature conditions (21°C). Their calculations predict a decreasing amount of He dissolved in the liquid CO<sub>2</sub> as the pressure decreases. The experiments reported above, which used a liquid CO<sub>2</sub>/He cylinder, were started at a tank pressure of 1650 psi, which should contain 4 mol % helium dissolved in the liquid CO<sub>2</sub> (~1 molecule in 20 is helium). Our final extractions (at 8000 and 10 000 psi) were conducted at relative constant tank pressure of 1300 psi, where the amount of helium in the liquid CO<sub>2</sub> is calculated to be 2.36 mol %. Therefore, the effect of decreasing the amount of helium dissolved in liquid CO<sub>2</sub> would be to enhance the solvent power of the fluid mixture from the liquid CO<sub>2</sub>/He during the SFE, similar to the density analogy noted above. Whereas this may partially account for the trend noticed at the higher extraction pressures, it cannot explain the considerable differences in the recorded oil solubilities (for example, the next to last extraction conducted with a liquid CO<sub>2</sub>/He source at 10 000 psi yielded an oil solubility of 5.47 wt %, and the last extraction at 8000 psi gave a 2.86 wt % oil solubility). It is also worth noting that had all of the extractions been conducted with a liquid CO<sub>2</sub>/He source, using all new cylinders pressurized to say 1700 psi, then the recorded solubility differences between neat CO<sub>2</sub> and helium-pressurized cylinders would have been even greater than those reported in this study. Therefore, we feel that the effect noted in this study is valid and should be of importance to analysts using SFE.

The layering or miscibility of binary gas mixtures above and below their respective critical temperatures at high pressures has been studied by several investigators.<sup>22,23</sup> The phenomenon is quite general, as noted by Tsiklis and Rott in an excellent review,<sup>24</sup> and the tendency toward phase separation or mutual miscibility

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of binary fluid pairs can be qualitatively predicted by a cohesive energy density approach.<sup>25</sup> However, despite the predicted immiscibility of He with CO<sub>2</sub> under the above conditions (which is the basis of using He as a pressurizing medium with liquid CO<sub>2</sub>), real experimental measurements show that there is a finite solubility of He in liquid CO<sub>2</sub>.<sup>21,26</sup> It is this factor which apparently accounts for the results reported in this study.

## CONCLUSIONS

The results presented in this study show that the small amount of helium entrained in liquid carbon dioxide is capable of reducing the dissolution power of supercritical CO<sub>2</sub>. The observed reduction in solute solubility resulting from this phenomenon should be appreciated by analysts utilizing SFE, particularly in optimizing extraction conditions and where method validation is required. Small quantities of He in SC-CO<sub>2</sub> can result in an incomplete extraction, particularly if the extraction conditions (time, volume, or mass of fluid used) are based on solute solubilities in pure

CO<sub>2</sub>. The above trend does not imply that helium-pressurized CO<sub>2</sub> cylinders and their attendant benefits cannot be utilized for analytical SFE. However, analysts should take precautions when utilizing solubility data determined in pure CO<sub>2</sub> with these fluid sources and the booster-type pumps utilized in this study.

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